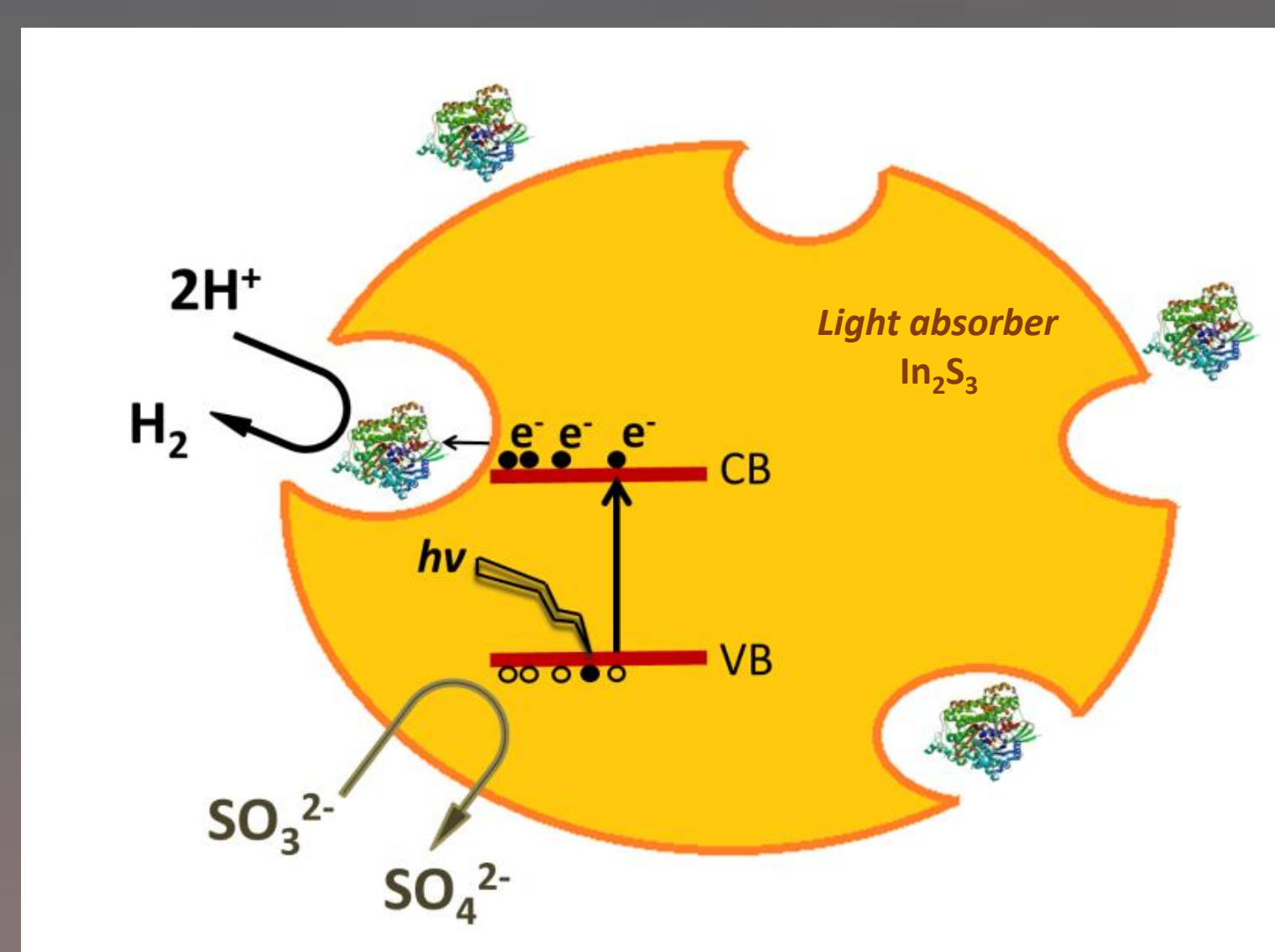


# In situ determination of photobioproduction of H<sub>2</sub> by In<sub>2</sub>S<sub>3</sub>-[NiFeSe] Hydrogenase from *D. vulgaris* Hildenborough using only visible light

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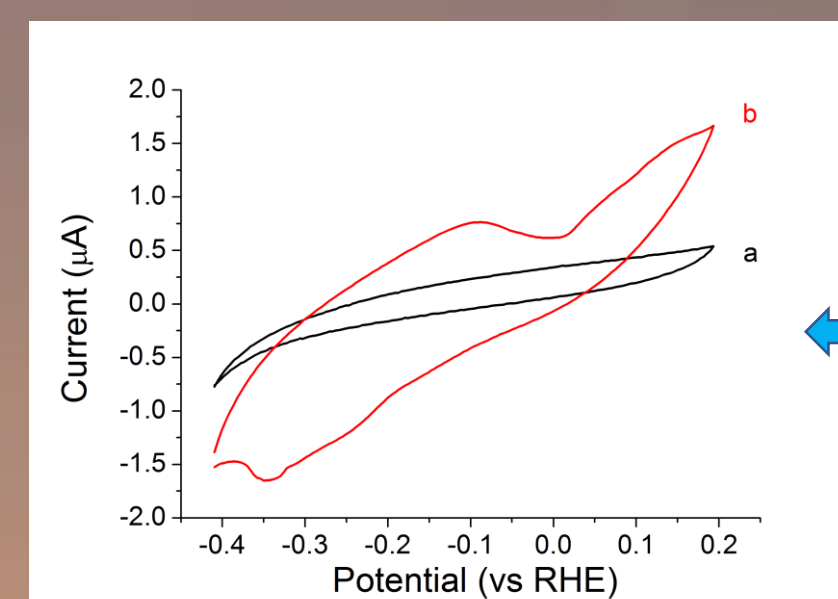
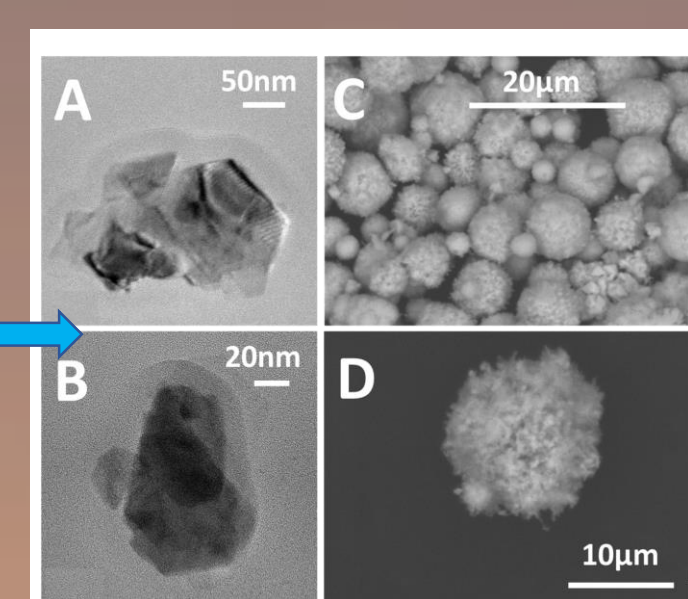
**Photocatalytic hydrogen evolution** is possible employing a hybrid system combining a solid-state visible light absorber, such as In<sub>2</sub>S<sub>3</sub> semiconductor, with the [NiFeSe]-Hydrogenase from *Desulfovibrio vulgaris* (Dv-SeHase).

The energy of the conduction band of In<sub>2</sub>S<sub>3</sub> is high enough to supply the hydrogenase with photoexcited electrons for H<sub>2</sub> production [1]. Direct electron transfer (DET) between the conduction band and the distal cluster of hydrogenase is aimed to obtain photocatalytic production of hydrogen. The photocatalytic activity of soluble form of the Dv-SeHase incubated previously with In<sub>2</sub>S<sub>3</sub> particles was measured by membrane-inlet mass spectrometry connected to an anaerobic vessel with no gas phase, under visible light irradiation [2].

## The In<sub>2</sub>S<sub>3</sub> light absorber characterization

### In<sub>2</sub>S<sub>3</sub> structure and shape

TEM images (A,B) show hexagonal nanocrystal shape particles ranging 50-100nm in diameter. SEM images (C, D) show flower like spherical particle aggregation with 2-15µm of diameter and high level of porosity.

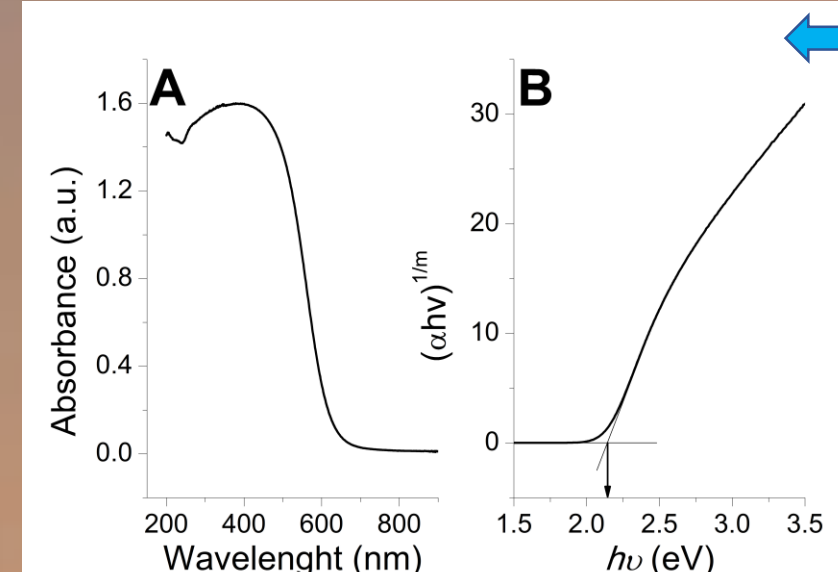


### Redox potential under irradiation

Electrochemical characterization of In<sub>2</sub>S<sub>3</sub> deposited on LDG electrode. A value of standard redox potential of ≈ -0,2 vs. RHE under irradiation (b) indicates that there is a fair overpotential to make possible the electron transfer to hydrogenase.

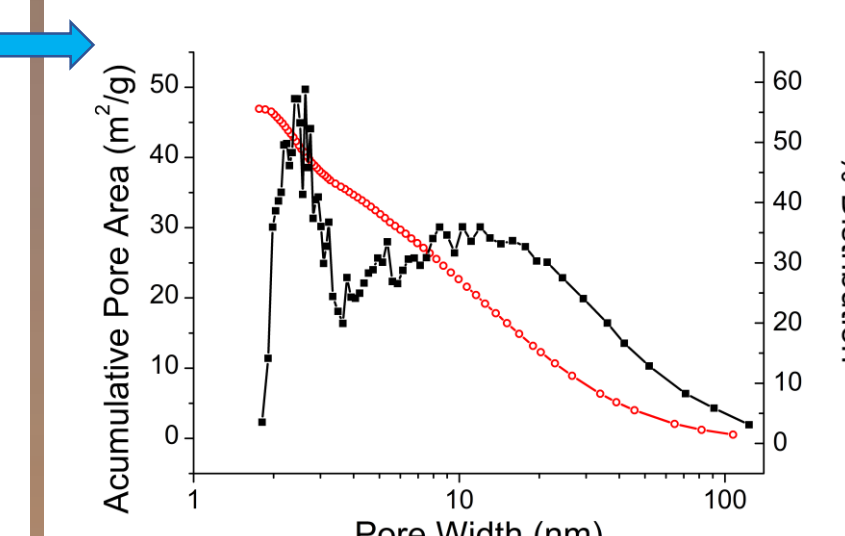
### The band-gap energy

Diffuse reflectance UV-Vis spectrum (A) and band-gap determination (B) show that In<sub>2</sub>S<sub>3</sub> absorbs in the range of 350-550nm with a band-gap of 2,1eV.



### Pore size for hydrogenase insertion

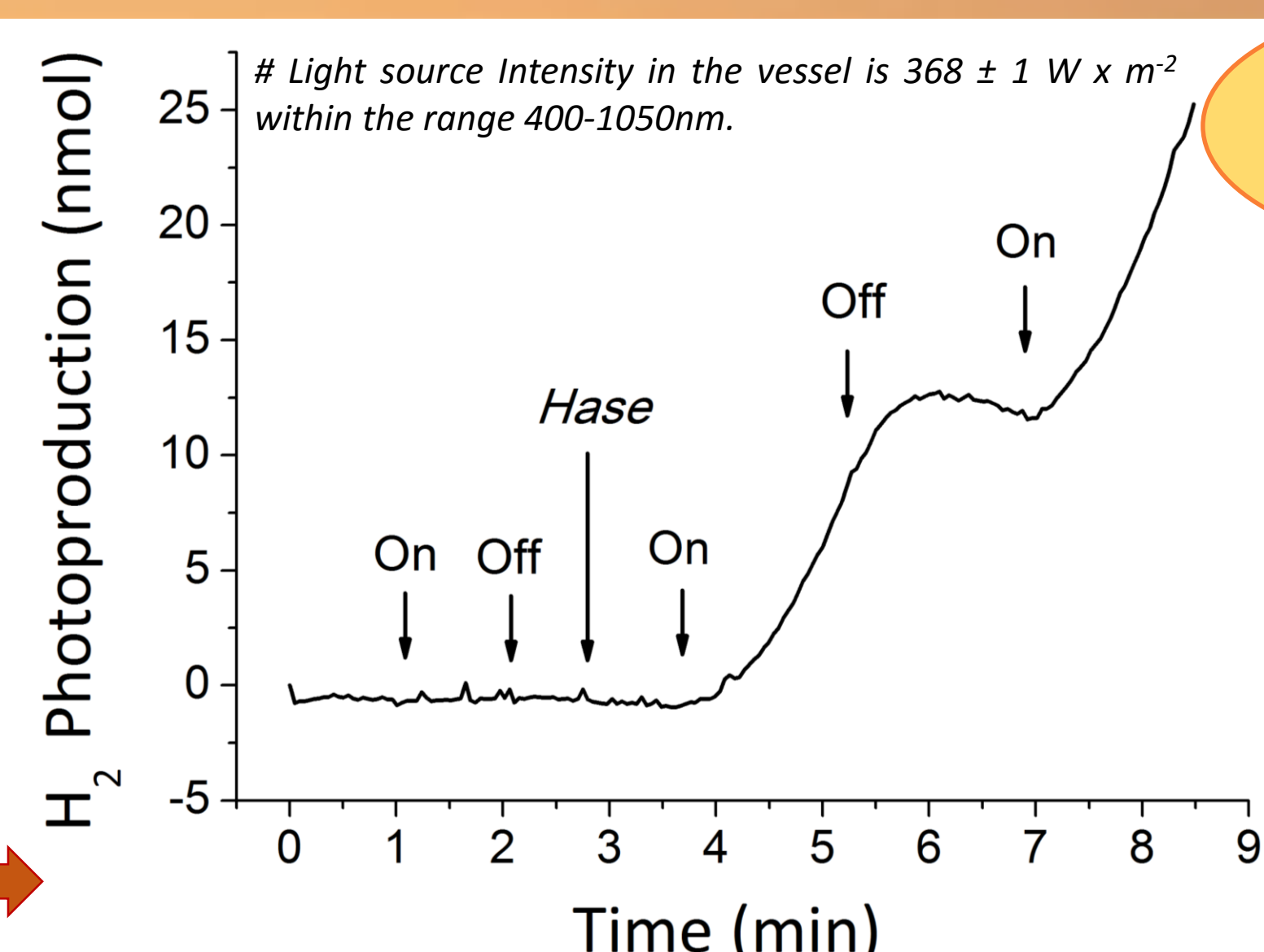
The specific area of the In<sub>2</sub>S<sub>3</sub> powder measured by BET method obtained a value of 40,6±0,3 m<sup>2</sup>/g and the average pore width was 16,5nm. The diameter for the Dv-SeHase molecules is around 5nm, which favor the insertion of the enzyme into the pores during incubation process.



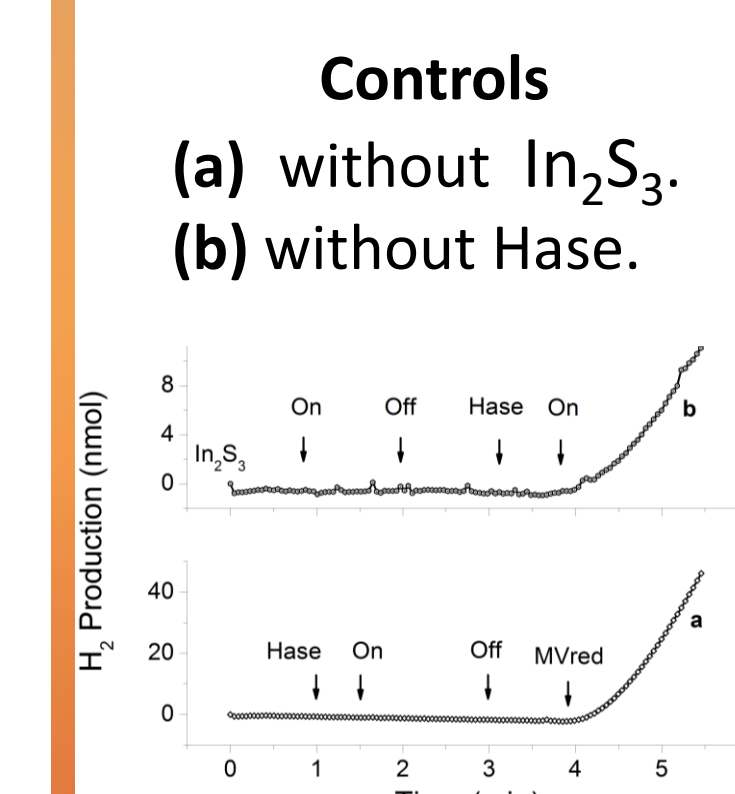
## The hybrid In<sub>2</sub>S<sub>3</sub> / Hase photocatalyst

### The Photocatalytic activity measurement

H<sub>2</sub> production is measured by mass spectrometry in 50mM Tris-HCl, pH 7, containing 0,2M sulfite, at 37°C. The Sodium sulfite was chosen as sacrificial agent. The experiment started with the reactor containing only In<sub>2</sub>S<sub>3</sub> and sulfite. The solution was irradiated from minute 1 to 2 with no production of H<sub>2</sub> during that time frame. Afterwards the Hase was injected inside the vessel. After 1 minute mixing the light was switched on. H<sub>2</sub> production was observed almost immediately. Switching off the light source interrupted the H<sub>2</sub> production.



Photoproduction of 292 U\*



### Sulfite effect

The effect of the presence of sulfite on the specific activity of the enzyme was tested using reduced methyl viologen 1mM concentration added in the solution. The specific activity with sulfite was 1140 ± 45 U\*. Whereas in absence of sulfite was 3763 ± 221 U\*. Sulfite decrease 3-fold the catalytic turnover of the Hase. In<sub>2</sub>S<sub>3</sub> does not affect to Hase.

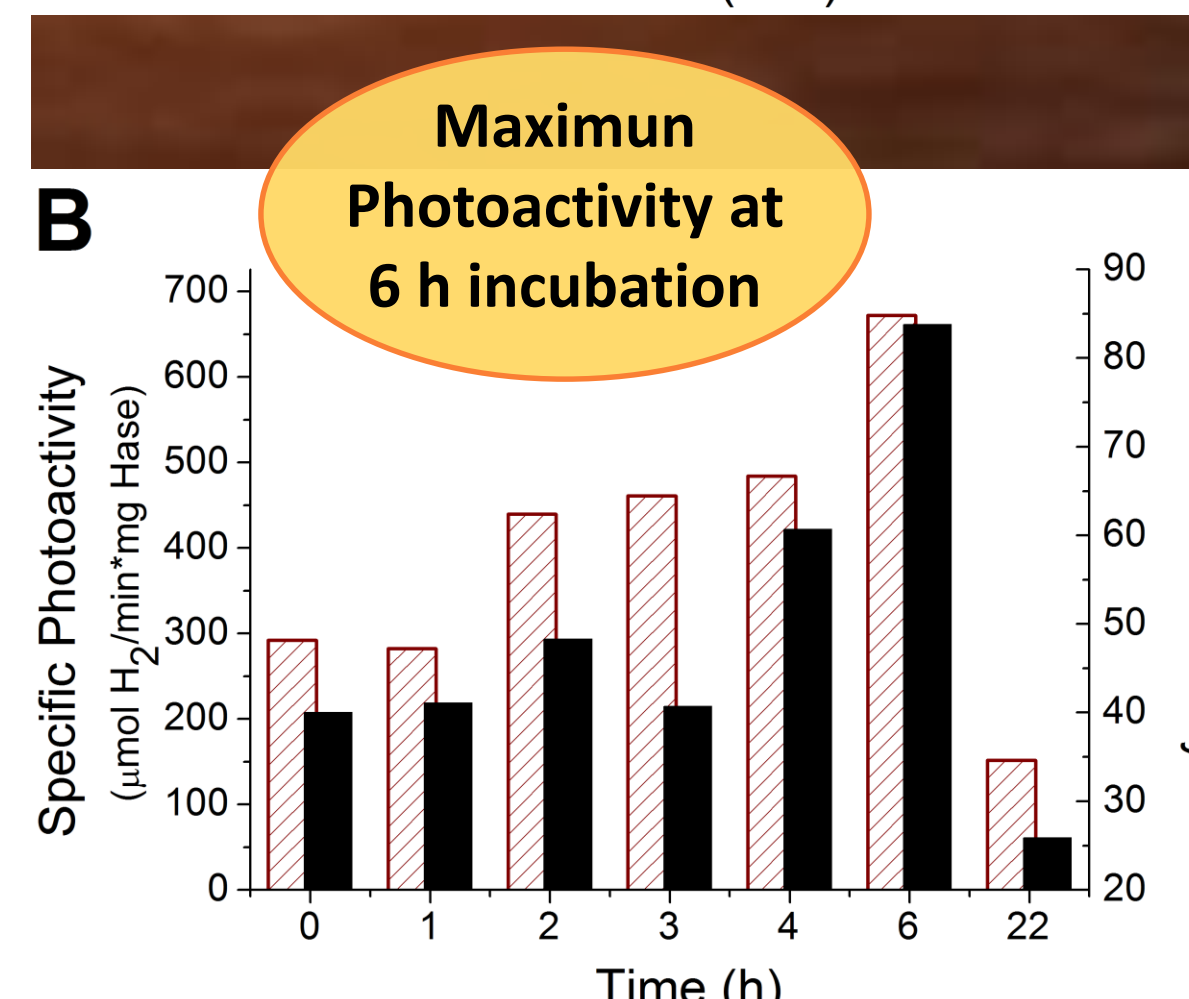
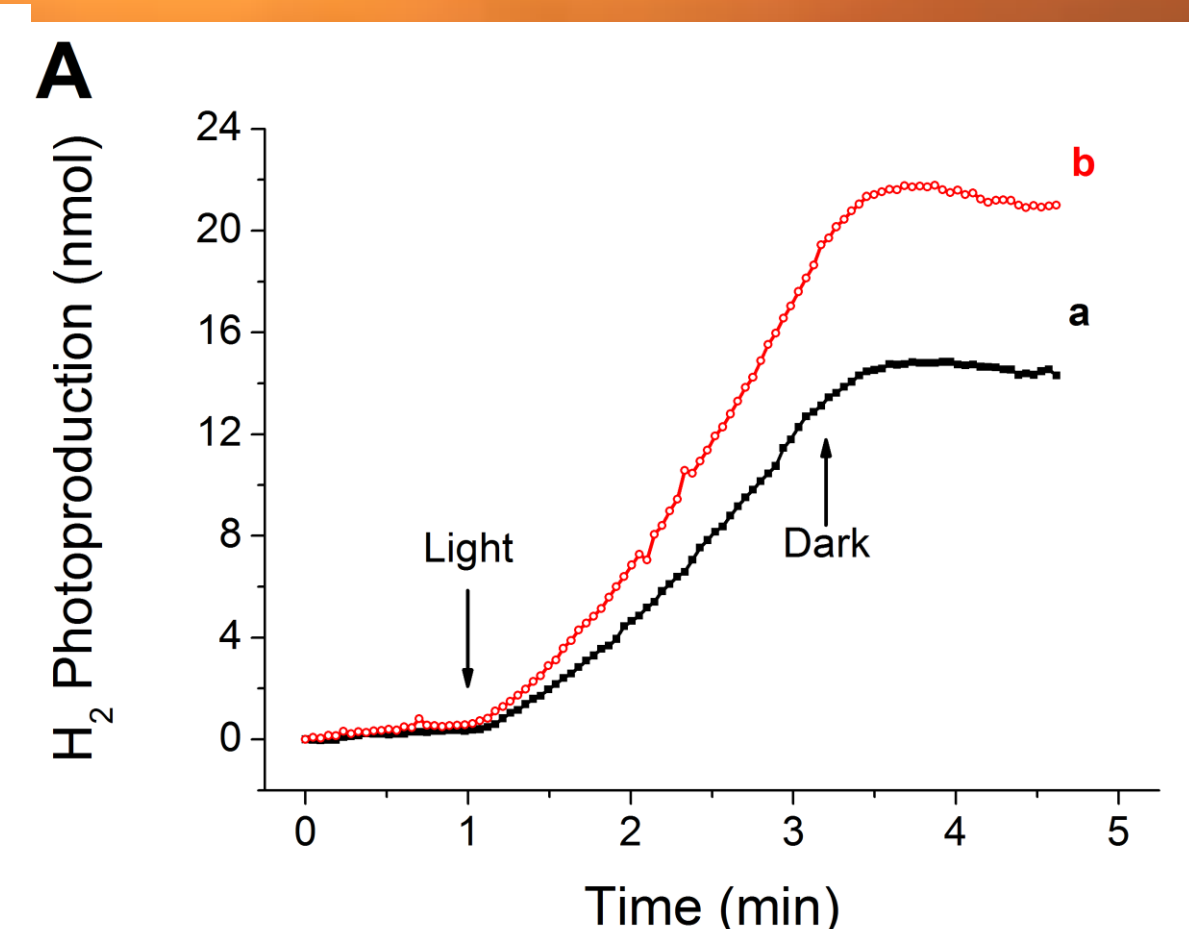
U\* [µmol H<sub>2</sub> x mg<sup>-1</sup> Hase x min<sup>-1</sup>]

## The incubation effect on hybrid In<sub>2</sub>S<sub>3</sub> / Hase photocatalysis

### Higher photocatalytic rate after incubation of Hase with In<sub>2</sub>S<sub>3</sub>

Figure A shows the comparison between an experiment with no previous incubation time (a) and another after 3 hours of In<sub>2</sub>S<sub>3</sub> and Hase suspension incubation at 4°C stirred at 60 rpm speed on a roller mixer. Higher photoproduction was achieved after incubation.

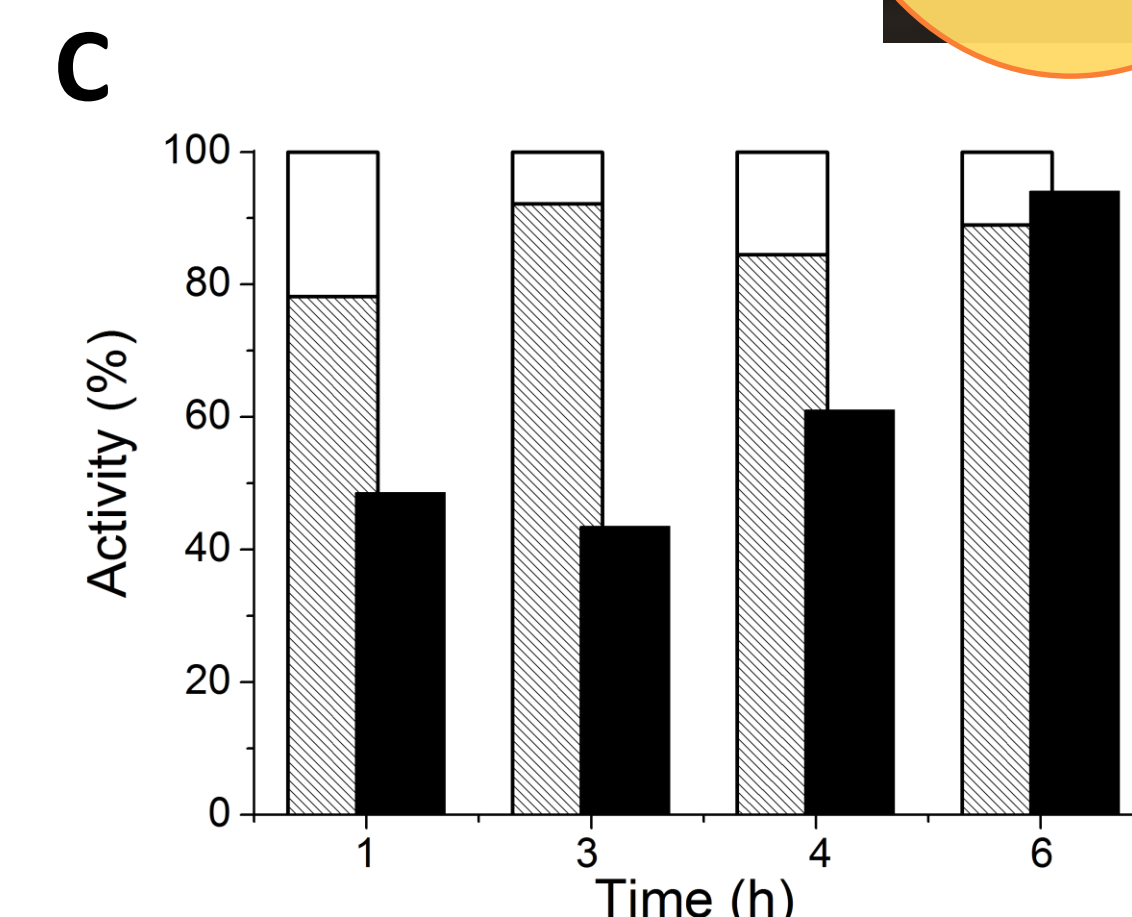
Figure B show the dependence of photoactivity on the incubation time (tested from 0 to 22 hours). A longer incubation yielded a higher photobioproduction of H<sub>2</sub>, with a maximum at 6 hours incubation. The striped bars show the specific photoactivity. The black bars shown the % of photocatalytic rate for each sample compared with the measured H<sub>2</sub> production rate driven by reduced methyl viologen instead of light. The efficiency of the photoexcited system with no previous incubation is 40%, whereas the 6-hour incubation sample yielded 84% H<sub>2</sub>-photobioproduction rate efficiency.



### Hydrogenase inserted into the pores of the light absorber

The enzyme ratios attached to the In<sub>2</sub>S<sub>3</sub> particles were measured by incubating the samples and then let to sediment under natural gravity during two hours. The supernatant liquid was then separated, and the sediment particles resuspended in fresh buffer. Figure C represents the % of H<sub>2</sub> production obtained with the supernatant fraction (white area in bars) and the In<sub>2</sub>S<sub>3</sub> particles fraction (grey area in bars).

The black bars are the % of photoactivity in the sediment fraction compared to the Hase activity measured in the same fraction with MV. After 6 hours incubation the 89% of the Hase was attached to the In<sub>2</sub>S<sub>3</sub> particles and 94% of it was photoactive.



94% of the Hase inserted into the pores of the In<sub>2</sub>S<sub>3</sub> is photoactive

## CONCLUSIONS

- Photoexcited electrons that populate the In<sub>2</sub>S<sub>3</sub> conductive band (CB) were transferred to the Hase's active site successfully, thus allowing the reduction of two protons to H<sub>2</sub>.
- 6-hours incubation sample yielded the highest H<sub>2</sub> – photoproduction rate efficiency: 89% of the Hase was inserted inside the pores of the In<sub>2</sub>S<sub>3</sub> particles and 94% of it was photoactive.

In<sub>2</sub>S<sub>3</sub> and *D. vulgaris* [NiFeSe] hydrogenase form an efficient hybrid photocatalyst for solar H<sub>2</sub> production

[1] Y. Xu, M. A. A. Schoonen, *American Mineralogist*, 2000, Vol. 85, page 543.

[2] Y. Jouanneau, B.C. Kelley, Y. Berlier, P.A. Lespinat and P.M. Vignais. *J. Bacteriol.* 1980, Vol.143, page 628.

[3] C. Tapia, S. Zacarias, I. A. C. Pereira, J. C. Conesa, M. Pita, A. L De Lacey, *Submitted*, 2016.

Thanks to Spanish MINECO CTQ2012-32448 Project and BES-2013-064099 Contract.